

CLAIMS

1. An enzyme mixture for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, and said second enzyme comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

2. The enzyme mixture of claim 1, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

3. The enzyme mixture of claim 2, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

4. The enzyme mixture of claim 1, wherein said second enzyme is a mutant DNA polymerase.

5. The enzyme mixture of claim 4, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

6. An enzyme mixture for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

7. An enzyme mixture for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme is a Taq DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

8. The enzyme mixture of claim 4, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

- 5 9. The enzyme mixture of claim 6, 7, or 8, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.
10. The enzyme mixture of claim 9, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G,
10 K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
11. The enzyme mixture of claim 6, 7, or 8, further comprising a PCR enhancing factor and/or an additive.
12. A kit for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, said second enzyme comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity, and packaging material therefore.
13. The kit of claim 12, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.
14. The kit of claim 13, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA
20 polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.
15. The kit of claim 14, wherein said second enzyme is a mutant DNA polymerase.
16. The kit of claim 15, wherein said mutant DNA polymerase is derived from a DNA
25 polymerase selected from the group consisting of: UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.
17. The kit of claim 16, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

- 5 18. A kit comprising an enzyme mixture for DNA synthesis, said kit comprises a first enzyme and a second enzyme, and packaging material therefore, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.
- 10 19. A kit comprising an enzyme mixture for DNA synthesis, said kit comprises a first enzyme and a second enzyme, and packaging material therefore, wherein said first enzyme is a Taq DNA polymerase, and packaging material therefore, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.
- 15 20. The kit of claim 12, 18, or 19, further comprising one or more components selected from the group consisting of: a deoxynucleotide, a reaction buffer, a PCR enhancing factor and/or an additive, a control DNA template and a control primer.
- 20 21. The kit of claim 15, 18, or 19, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.
- 25 22. The kit of claim 21, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
23. A method for DNA synthesis comprising:
 - (a) providing an enzyme mixture, said enzyme mixture comprising a first enzyme comprising a DNA polymerization activity, and a second enzyme comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity; and
 - (b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.
- 30 24. The method of claim 23, wherein said nucleic acid template is a DNA or an RNA molecule.

25. The method of claim 24, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

26. The method of claim 25, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

27. The method of claim 24, wherein said second enzyme is a mutant DNA polymerase.

28. The method of claim 27, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

29. The method of claim 27, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

30. A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a wild type Pfu DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

31. A method for TA cloning of DNA synthesis product comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a Taq DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity;

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and